

EXTRACTION EFFICIENCY OF ORGANICS FROM MARS REGOLITH ANALOGUE SAMPLES. L.C.Welzenbach¹, S. Nicoara¹, M. Kershaw¹, M.M. Grady^{1,3}, R.C. Greenwood¹, M.D. Fries², F.M. McCubbin², C.L. Smith^{3,4}, A. Steele⁵, ¹The Open University, Milton Keynes, UK (lwelzenbach@rice.edu), ²NASA-Johnson Space Center, Houston TX, ³The Natural History Museum, London UK, ⁴The University of Glasgow, UK, ⁵Geophysical Laboratory, Carnegie Institution of Washington DC

Introduction: The Mars 2020 rover mission has collected and cached samples from the martian surface for possible retrieval and subsequent return to Earth. Mars Sample Return (MSR) materials are believed to contain organic compounds [1] that will be thoroughly examined to test the hypothesis of the existence of past or present life on Mars. Post-mission analyses will depend on the development of a set of reliable sample handling and analysis procedures that cover the full range of materials, which may or may not contain evidence of past or present martian life [2].

MSR presents unique challenges for the collection, containment, transport, curation and processing of samples [2]. Foremost in the processing of returned samples are the closely paired considerations of life detection and Planetary Protection. In order to achieve MSR science goals, reliable analyses will depend on overcoming some challenging signal/noise-related issues where sparse martian organic compounds must be reliably analyzed against the contamination background. While reliable analyses will depend on initial clean acquisition and robust documentation of all aspects of developing and managing the cache [2], there needs to be a reliable sample handling and analysis procedure that accounts for a variety of materials which may or may not contain evidence of past or present martian life.

Any set of sample handling and examination procedures would include a phased approach that would be accepted by the community to preserve the bulk of the material, but provide unambiguous science data that can be used and interpreted by various disciplines. Foremost is the concern that the initial steps would ensure the pristine nature of the samples.

Preliminary, non-invasive techniques, such as computed X-ray tomography (XCT), have been suggested as the first method to interrogate and characterize the cached samples, without altering them significantly [2,3]. XCT has been shown to minimally alter samples for most investigations [4], yet is documented to impact others [5]. One of the main aims of the present study is to identify, and if possible, quantify any of these effects.

Identifying Organics in MSR: Carbonaceous materials may comprise a component of Mars regolith and are of high scientific importance in the light of MSR scientific goals related to understanding the physiochemical history of the martian surface and the search for evidence of past or present life. In terms of the amount of carbon present, Eigenbrode et al. (2018) [6] reported that a “refractory carbon” component within the regolith, possibly delivered through carbonaceous meteorite infall, can be expected to be at or below the ppb

level, taxing the capabilities of current analytical laboratories. Because of this, identification and quantification of the types of carbonaceous compounds present in the samples is critically important. In addition, the extent of potential alteration of the returned MSR samples needs to be determined. Some of this alteration may take place during preliminary examination by XCT rather than on the surface of Mars. Deconvoluting such effects is critically important to achieving MSR scientific goals.

Selection of Mars-relevant organic analogues- Analysis of Mars 2020 organic compound analogues draws on the work undertaken by the Mars 2020 Organic Contamination Panel (OCP) in identifying appropriate native martian and contaminant compounds [3]. In their final report, the OCP produced two extensive lists of organics of interest; Tier 1 and Tier 2 compounds. The “Tier 1” list of compounds are described as those “...likely to be most important to the science goals of the mission” and in which the mission is recommended to control to within 1 ppb concentration per compound. These target organics would be both a Mars relevant signal and equivalent potential contaminants that could be measurable by current analytical techniques.

Experimental approach- Selected “Tier 1” organic compound will be used in an experiment to simulate a preliminary examination of a Mars return sample cache tube. The goal is to identify any alteration of organic materials associated with exposure to ionizing radiation during XCT examination. Eight compounds were selected to include one representative from each major compound class that are scientifically important contaminants and potential biosignatures. They are also reasonable approximations of major contributors from carbonaceous chondrite meteorites, either as infall onto Mars [6], or as material directly sampled on an asteroid by OSIRIS REx and Hayabusa2. These include sugar (glucose), an amino acid (glycine), a nucleic acid (adenine), a lipid (palmitic acid), a hydrocarbon (heptacosane), a nitrogenous compound (urea), a hydroxy carboxylic acid (pyruvic acid), and a polycyclic aromatic hydrocarbon (PAH, naphthalene). All of the selected compounds are isotopically labeled (D, ¹⁵N, ¹³C) so that they are easily discernible in post-exposure analyses from contaminants that may be introduced during all stages of the experiment, but also to potentially identify any by-products of x-ray ionizing radiation.

Mars Analogue Sample Experiments: Four sets of experimental cache tubes were assembled at NASA-JSC. Each set includes 9 samples, 8 of which are doped individually with Mars-relevant organics and one blank. Titanium 6Al/4V

(Grade 5) tubes of similar diameter and wall thickness to Mars 2020 cache tubes were loaded cleanly (under ultrapure nitrogen) with Mars analogue regolith simulant (MRS-1) [7] that was hand-mixed with an individual powered aliquot of isotopically labelled organic compound. Samples were capped, enclosed individually in Teflon bags, then stored frozen in a -30° freezer until scanning by XCT with the Nikon XTH 320 at NASA-JSC. Three of the four sets were imaged at conditions set in previous work [8], ranging from the minimum time and power needed to resolve contents through the titanium tube to the maximum energy and scan time to produce a high resolution image of mm-sized regolith particles. The shortest scan was less than an hour and the longest scan was seven hours. Samples were returned to the freezer immediately following the scan and then shipped to the Open University (OU) for analyses.

Sample Processing for organic analysis-The initial experimental design was to mix all of the organic compounds together with the regolith analogue and analyze them as one. Early attempts to analyze for all the organic compounds together by LCMS proved to be problematic due to the diversity of solvents needed to extract each compound. Additional issues were compatibility with the mobile phase and column polarity. This led to poor elution, and resulted in detection problems and hindered calculation of the limit of quantitation. In order to have the highest confidence in the identification of original organic compound and any radiation byproducts, each cache tube now only contains one compound and so extraction and analysis are not compromised by the presence of additional simulants. This approach not only eliminates cross-compound interactions but also provides a strong basis for identifying contamination introduced after the regolith/simulant mixtures were assembled. An important consideration is the ability to extract the compound in its entirety along with any by-products from the regolith that may have been produced from the regolith.

Efficiency of Extraction (E^2)- Organic compound extraction and analysis method development is the recent focus of this study in order to establish the best instrumental and analytical procedures to evaluate both the limit of detection and provide optimal quantitation. The various organic compounds are being analysed by LCMS and GCMS (Table 1) as determined by polarity and compatibility with the instruments' column and mobile phase. Test samples using palmitic acid and naphthalene were assembled at the OU using non-labelled compounds mixed with Mars regolith analogue in the same sample mass (~0.2g of compound in 4g of simulant) as the already exposed samples.

Using the appropriate solvents, each of the compounds were extracted and processed for LCMS and GCMS. Samples of palmitic acid and naphthalene were extracted, derivitized (for LCMS), diluted and run on the Thermo Accela LC coupled to a Thermo TSQ Quantum MS with a Phenomenex Omega PS column (150x2.1 mm, 2µm particle size) using an

Table 1: List of organic compounds and the system on which they will be analyzed.

Analyte	Solvent	logP	LCMS	GCMS
Naphthalene	Hexane	3.3		x
Heptacosane	Hexane	14.2		x
Palmitic acid	Hexane (GC); 13% H ₂ O in Methanol (LC)	6.4	x	x
Urea	10% H ₂ O in AcCN	-1.4	x	
Adenine	10% H ₂ O in AcCN	-0.1	x	
Glycine	35% H ₂ O in AcCN	-3.2	x	
Pyruvic acid	100% H ₂ O	0.3	x	
Glucose	10% H ₂ O in AcCN	-2.9	x	

isocratic mobile phase of 13:87 H₂O:MeOH with 0.1% acetic acid, and in hexane for the Agilent GCMS Phenomenex Zebtron FFAP column. A calibration series of known concentrations of palmitic acid was prepared. Prior to injection, all samples were combined with an identical quantity of palmitic acid-d31 internal standard. The peak area ratio of the non-labelled palmitic acid and the labelled internal standard was calculated for both the extracted samples and the standards. Quantitation was performed by comparing the ratio for the extracted sample with a calibration curve constructed by plotting the peak area ratio against standard concentration.

Discussion: From preliminary results, comparison of the concentration of the extracted palmitic acid with standards gives values for the E^2 for palmitic acid of 74 and 77% of the original mass for GCMS and LCMS respectively. GCMS evaluation of E^2 for naphthalene indicates a value of 67%. A second extraction of each analyte with subsequent runs on both systems may provide an idea of how much remaining analyte can be extracted. Not only would it provide more information on the effectiveness of the extraction method, but may reveal the choice and limit of solubility for the solvent. A second extraction would likely release many times less material but, if the LCMS or GCMS peak area is at all similar between the first and second, we would look to the solvent type or concentration. Similar efficiency of extraction experiments are in process for the remaining 5 compounds.

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